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Emerging technology to develop novel red winemaking practices: An overview☆

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abstract

Nowadays, making modifications to traditional practices and/or adopting novel processing technologies is of great interest in order to fulfill consumers expectations towards food products characterized by convenience, variety, adequate shelf-life and caloric content, healthy properties, reasonable cost, and environmental sustainability. In this perspective, the role of emerging technologies in winemaking is addressed towards reduced production time, optimized resources and spaces, extraction of high nutraceutic components through mechanical effects, high energy efficiency, extended shelf-life, lowering SO_2 addition and its final concentration. This paper is the outcome of an extensive and comprehensive literature review describing, by an integrated approach, the main characteristics and applications of three emerging technologies (US, MW, and PEF) alternative to the traditional winemaking processes. Their advantages related to the safety aspects of wine, such as the ability to improve nutraceutic and sensorial features are also described.

Industrial relevance: The food industry is currently interested in a variety of novel production and emerging technologies that may result in economical and quality products. This review shows as numerous researches have strongly demonstrated the great benefits of new emerging technologies, such as PEF, US, and MW, into the oenological industry, either increasing compounds extraction during maceration of the must or accelerating stabilization stage in the wine. Emerging technologies could offer better products to consumers with added value in terms of nutritional or sensorial characteristics, and guarantee higher profit for the industry, even reducing process time and use of natural resources, such as energy, water, and chemicals.

Contents

Abbreviations: US, ultrasound; MW, microwave; PEF, pulsed electric field; GC–MS, gas chromatography–mass spectrometry; PPO, polyphenol oxidase; UAE, ultrasound assisted extraction; SE, Soxhlet extraction; AAR, antiradical activity; TPC, total phenolic content; MWP, process variables microwave; MAE, microwave-assisted extraction; CSE, conventional solvent extraction; HPTE, high pressure and temperature extraction; CI, color intensity; AC, anthocyanin content; TPI, total polyphenols index; HPLC, High Performance Liquid Chromatography; HVED, high voltage electric discharges.

☆ In memory of Professor Ennio La Notte (University of Foggia, Italy).

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1. Introduction

Winemaking comprises a diverse set of factors that play a crucial role during the transformation of grapes to wine. The most important factors generally considered by winemakers include vineyard management, grape quality, winemaking practices, and the proper use of commercial selected yeasts and lactic acid bacteria. Increasing consumer demand for food and beverage with a highest added value (Azzurra & Paola, 2009; Kiesel, McCluskey, & Villas-Boas, 2011) led to the development of new alternative processes (Knorr et al., 2011) to enhance or substitute conventional winemaking techniques.

Recently, emerging technology, such as ultrasound (US), microwave (MW), and pulsed electric field (PEF), have been tested in order to develop novel red winemaking practices (Table 1), with different aims, such as increasing the product throughput and reducing the production run, that enable to the winery of optimizing resources and spaces, sales and turnover, by improving the cash flow (Martín & Sun, 2013). Moreover, they allow an environmentally sustainable management, in term of energetic efficiency (Toepfl, Mathys, Heinz & Knorr, 2006; Sun, 2014), and minor use of water and pollutants (such as the flowing detergents, adjuvants, etc.) (Clodoveo, 2013).

Two other aspects are very important to highlight: US, MW, and PEF are characterized by mechanical effects able (1) to increase the extraction of nutraceutic valuable components into the resulting wine, thus improving the quality and healthy value of the product (Vilkhu, Mawson, Simons, & Bates, 2008) and (2) to disrupt or damage the cellular membrane of either autochthonous yeasts and bacteria in grape must before primary fermentation or spoilage microorganisms in wine, thus markedly reducing the addition of SO_2, as antiseptic agent, during winemaking (National Advisory Committee on Microbiological Criteria for Foods, 2006; Cui, Lv, Liu, & Wang, 2012; Martín & Sun, 2013; Aneja, Dhiman, Aggarwal, & Aneja, 2014; Carew, Close, & Dambergs, 2015). Nevertheless, since SO_2 is commonly used in vinification also for its antioxidant properties (Santos, Nunes, Saraiva, & Coimbra, 2012) and because it can affect the activity of some grape enzymes which promote the loss of quality of the juice and its derivatives (e.g. polyphenoloxidases, including tyrosinase and peroxidase) (Popescu, Postolache, Rapeanu, Bulancea, & Hopulele, 2010), its addition cannot be completely avoided. However, the decrease of the final SO_2 concentration in wine is highly recommended, since it might cause sensitivity reactions such as dermatologic, respiratory, or gastrointestinal symptoms (Vally & Misso, 2012). Besides the direct application to grape must or wine, MW might be successfully used for the microbial sanitization of barrels (González-Arenzana et al., 2013) and US and PEF for assisting the wine aging process (Martín & Sun, 2013).

2. Conventional red winemaking systems

The basic steps of red wine production is shown in Scheme 1. Red winemaking is firstly based on the maceration, which is the process of soaking crushed grape skins, seeds, and, eventually, stems and whose management is one of the most critical aspects; indeed, it is fundamental to extract the colorful and tannic components into wine, but it should not be too prolonged because it could cause an excessive bitter and astringent taste in wine of some varieties, often not well appreciated by consumers (Ribereau-Gayon, Dubourdieu, Doneche, & Lonvaud, 2000; Pinelo, Arnous, & Meyer, 2006; Sokolowsky, Rosenberger, & Fischer, 2015).

After the chosen maceration period, which can depends on the aging purpose of wine, the only partly fermented must is separated from skins and seeds by using a press and the alcoholic fermentation can be completed to almost eliminate sugar residue in the wine (Ribereau-Gayon et al., 2000).

Subsequently, wine may undergo malolactic fermentation, a biological decarboxylation carried out by lactic acid bacteria (species of the genera *Lactobacillus*, *Pediococcus* and *Oenococcus*), able to confer a rounder and fuller mouthfeel to the wine; it can take place spontaneously, if favored by different parameters (such as temperature, concentration of SO_2 and nutrients), or by inoculating selected lactic acid bacteria strains (mainly belonging to *Oenococcus oeni* species).

The last phase is the wine aging, that generally consists of different steps (Ribereau-Gayon et al., 2000) including maturation (oxidative aging) and bottling (reductive aging). Moreover, clarification processes and storage in oak barrels (which requires considerable time and financial investment), might be carried out; if the young wine has a lot of grape tannins, the aging in barrels and then in bottles aims to reduce the strength and bitterness of these tannins and rounds out the flavors of the resulting wine (Martín & Sun, 2013).

3. Some new technologies applied to winemaking

3.1. Principles and mechanism of US

Ultrasound is a relatively low-cost, non-hazardous, and environmental friendly technology, commonly used in the food industry (Mason, Paniwnyk, & Lorimer, 1996). Ultrasounds (i.e., mechanical waves at a frequency above the threshold of human hearing) can be divided into three frequency ranges; power ultrasound (16–100 kHz), high frequency ultrasound (100 kHz–1 MHz), and diagnostic ultrasound (1–10 MHz). These sound waves are transmitted through any substance, solid, liquid or gas, which possesses elastic properties and travel either through the bulk of a material or on its surface at a speed depending on the nature of the wave and propagating material (Jambrak, 2011). The fundamental effect of ultrasounds on a flowing fluid is to impose acoustic pressure in addition to the hydrostatic pressure already acting on the medium. The acoustic pressure is a sinusoidal wave dependent on time (t), frequency (f) and the maximum pressure amplitude of the wave, $P_{a,max}$, which is directly proportional to the power input of the transducer:

$$P_a = P_{a,max} \sin(2\pi ft)$$

If the treatment is characterized by higher intensities, the local pressure in the expansion phase of the cycle falls below the vapor pressure of the liquid, causing tiny bubbles to grow producing new cavities due to the tensioning effect on the fluid and the negative transient pressures within the fluid. Within a critical size range the oscillation of the bubble wall matches that of the applied frequency of the sound waves causing the bubble to implode. As shown in Fig. 1, the compression and rarefaction of the medium particles and the consequent collapse of the bubbles determines a phenomenon called cavitation. During the implosion, very high temperatures (circa 5000 K) and pressures (circa 2000 atm) are reached. The implosion of the cavitation bubble also results in liquid jets up to 280 m/s. The resulting shear forces break the cell envelope mechanically, producing turbulence in the cavitation zone. Since the frequency is inversely proportional to the bubble size, low frequency ultrasound (that is, power ultrasound 16–100 kHz) generate large cavitation bubbles resulting in higher temperatures and pressures in the cavitation zone.

The combination of factors such as heat, pressure, and turbulence, is used to accelerate mass transfer in chemical reactions, to create new

Table 1
Summary of findings from the literature review.

	Types of matrix	Conditions	Process/effect	Treatment performed	References
Ultrasound	Grape	60 W/cm^2 and 20 kHz for 15 s	Improvement of the disruption of the cell walls and increase of the concentration of phenolic compounds in the juice	Laboratory scale	Mokkila et al., 2004
	Grape juice and wine	20-75 W and 24 kHz	Increase of the phenolic compounds (anthocyanins)	Laboratory scale	Vilkhu et al., 2008
	Grape juice	20 kHz constant for 10 min and pulse durations of 5 s on and 5 s off	Increase of the anthocyanins, color values and color index	Laboratory scale	Tiwari et al., 2010
	Red grape and wine	20 kHz frequency. Grape treatment for 3 and 5 min at the amplitude of 90%; wine treatment for 5 min at the amplitude level of 80%	Improvement of the extraction of polyphenolic substances, with a reduction in the duration of classic maceration	Laboratory scale	Ferraretto et al., 2013
	Destemmed red grape	37 kHz frequency, power effective 150 W, 0.5 W/cm^2 intensity, for 15 min	Improvement of the extraction of all phenol classes, especially anthocyanins and increase of the sensory characteristics	Laboratory scale	Coletta et al., 2013
	Wine lees	20 kHz frequency, power effective 200 W, for 3 min in continuous sonication	Extraction of cellular components during the aging on the lees	Laboratory scale	Cacciola et al., 2013
	Grape marc	25 kHz frequency, 300 W power effective, for 2.5, 5, 10, 20, 30, 40, 50, 60 and 80 min in continuous sonication	Increase of the extraction rate and isolation of novel potential bioactive components from by-products	Laboratory scale	Tao et al., 2014a, 2014b
	Must and wine	48 kHz frequency, for 10 min	Improvement of the extraction of aromatic chemicals and extraction efficiency	Laboratory scale	Cocito et al., 1995
	Grape	40 Hz frequency, for 20 min	Improvement of the extraction of aromatic precursors and compounds during maceration on the skins	Laboratory scale	Cui et al., 2012
	Grape	35 kHz frequency, 2000 W power, for 15 min continuous sonication	Increase of the amount of phenolic compounds in red wine	Laboratory scale	Tudose-Sandu-Ville et al., 2012
	Wine	20 kHz frequency	Reduction of the SO_2 addition	Laboratory scale	Jiranek et al., 2008
	Reds grape and grape seeds from winemaking by-products	24 kHz frequency, 200 W power	Recovery of tartaric and malic acid from red and white variety grapes by-products	Laboratory scale	Palma & Barroso, 2002
	Red grape pomace by-products	25 kHz frequency, at 300 W and temperature 20 °C for 60 min	Improvement of the efficiency of the antiradical activity and of the extraction yield of phenolic compounds	Laboratory scale	Drosou et al., 2015
Microwave	Must	300 W microwave power, for 60s	Shortening of the maceration time and improvement of polyphenols extraction	Laboratory scale	Carew et al., 2013
	Pinot noir grape	400 W power, for 7 min	Reduction of the SO_2 addition	Laboratory scale	Carew et al., 2014
	Red wine	420 W power, irradiation duration 5 min	Shortening of the aging process and improvement in the organoleptic quality	Laboratory scale	Zheng et al., 2011
	Grape seeds	800 W power, 2450 MHz frequency, intermittent microwave power for 30 s/min	Improvement of the overall wine quality	Laboratory scale	Krishnaswamy et al., 2013
	Chinese herb (*Radix puerariae*)	255 W microwave power, for 6.5 min	Improvement of the flavonoids extraction	Pilot scale	Wang et al., 2013
	Oak wine barrels	3000 W power, for 3 min	Decrease of the microbial cell density in oak barrels	Laboratory scale	González-Arenzana et al., 2013
	Grape wastes	60 W power, for 60 min	Increase of the efficiency of phenolics extraction (in particular for t-resveratrol)	Laboratory scale	Casazza et al., 2010
	Grape seeds and skin	373.15 W microwave power, for 59 s	Increase of the extraction of bioactive compounds	Laboratory scale	Medouni-Adrar et al., 2015
Pulsed electric field	Apple slices	500 V/cm, 1000 pulses, 100 μs duration	Modification of the tissue structure and permeability	Laboratory scale	Jemai & Vorobiev, 2002
	Listeria monocytogenes	Pulse frequency of 1 Hz, pulse width of 2000 μs and 28 kV/cm electric field strengths	Inactivation	Laboratory scale	Álvarez et al., 2003
	Grape skin	Electric field strength from 2 to 10 kV/cm, (0.4–6.7 kJ/kg) energy consumption	Improvement of the maceration process	Pilot scale	López et al., 2008a, 2008b
	Red wine	Fifty pulses at a frequency of 122 Hz, 5 kV/cm electric field strength (total specific energy: 3.67 kJ/kg)	Reduction of maceration time	Laboratory scale	Puertolas, Lopez, Condon, Alvarez and Raso, 2010; Puertolas, Saldaña, Condon, Alvarez and Raso, 2010; Puértolas et al., 2010
	Grape	Fifty pulses at a frequency of 1 Hz and electric field strengths of 5 kV/cm with 1.8 kJ/kg energy, and 10 kV/cm with 6.7 kJ/kg energy	Increase of the amount of polyphenols in the final wine and shortening of the wine-oaking period	Pilot scale	López et al., 2008b
	Red wine	31 kV/cm electric field strengths 3 μs of pulse duration	Increase of the color intensity, anthocyanin content and of total	Bench scale continuous system	Abca & Akdemir Evrendilek, 2014

(continued on next page)

Table 1 (*continued*)

Types of matrix	Conditions	Process/effect	Treatment performed	References
	and 500 pps of frequency	polyphenolic index		
Grape	20 µs pulses, 7.4 kV/cm electric field strengths, and 400 Hz frequency	Improvement of the overall wine quality	Laboratory scale	López-Giral et al., 2015
Must	30 kV/cm electric field strengths	Reduction of the SO_2 addition	Laboratory scale	Garde-Cerdan et al., 2008
Must and wine	29 kV/cm electric field strengths, 1 Hz frequency, 186 kJ/kg energy	Inactivation of the spoilage microorganisms in must and wine	Laboratory scale	Puértolas, López, Condón, Raso and Alvarez, 2009
Red wine	29 kV/cm electric field strengths, 186 kJ/kg energy	Alteration of the resistance of different wine spoilage microorganisms	Pilot scale	González-Arenzana et al., 2015
Red wine	20 kV/cm electric field strengths	Inactivation of microorganisms present in the wines before bottling	Laboratory scale	Delsart et al., 2015
Grape by-products	9 kV/cm electric field strengths, 2 Hz frequency, 10 kJ/kg energy	Increase of the antioxidant activity of grape by-products	Laboratory scale	Corrales et al., 2008
Fermented grape pomace	13.3 kV/cm electric field strengths, 0–564 kJ/kg energy	Increase of the extraction efficiency of phenolics compounds	Laboratory scale	Barba et al., 2015
Fermented grape pomace	12 kV/cm electric field strengths, 18 kJ/kg energy	Efficient and selective extraction of total anthocyanins	Laboratory scale	Brianceau et al., 2015

reaction pathways, to break down and dislodge particles or even to generate different products from those obtained under conventional conditions (Feng, Barbosa-Cánovas, & Weiss, 2011). The use of ultrasounds in industrial processes requires two main factors: a liquid medium (even if the liquid element forms only 5% of the overall medium) and a source of high-energy vibrations (Patist & Bates, 2008). The interaction of acoustic energy with a food is mainly substantiated through a liquid medium because the cavitation and cavitation-induced physical and chemical actions play an important role in the food quality alterations in an ultrasound-processed food (Luque-Garcıa & De Castro, 2003; Wang & Weller, 2006; Cravotto et al., 2008).

The ultrasound process parameters are essentially amplitude, pressure, temperature, viscosity, and concentration of solids; the extraction yield and/or rate is a function of the energy (kW) and the intensity (W/cm^2). A general relationship between flow rate and energy for several ultrasonic applications is reported in Fig. 2. Both energy and intensity are independent of scale and thus any ultrasonic process will be scalable using these two parameters.

3.1.1. US in red winemaking process

3.1.1.1. Effect of US on reducing winemaking time and aging. One of the effects of ultrasound useful to reduce the winemaking time is its ability to promote the breaking of the cell, improving the matter transfer. In particular, ultrasound can lead to a permeabilization of cell membranes to the intra-cellular substances, and it can reduce the selectivity of the cell membranes, significantly. The mechanical activity of US supports the diffusion of solvents into the tissue, speeding up the process of soaking crushed grapes.

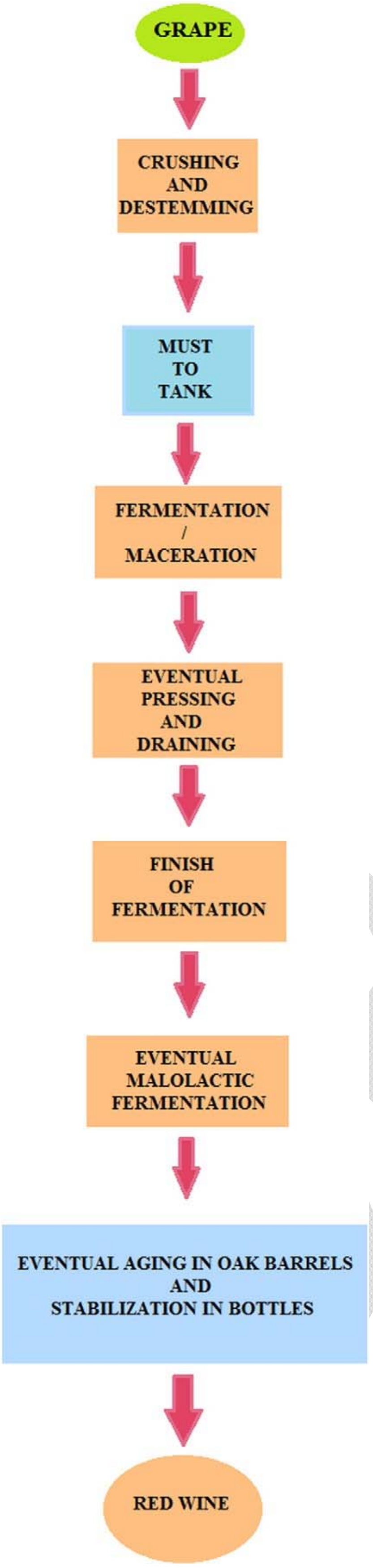
Shiraz, Merlot, and Negroamaro juices and wines with a higher content in anthocyanins, which is regarded as positive to produce high-quality wines, have been obtained employing US maceration technique without modifying basic oenological parameters such as pH, acidity, alcohol, and sugar content (Coletta et al., 2013; Vilkhu et al., 2008). Ferraretto, Cacciola, Batllo, and Celotti (2013) studied a possible application of US in the wine industry with the aim to fasten some reactions (thermodynamically possible, but kinetically slow) required in the winemaking process. Firstly, they found an improvement in the extraction of phenolic compounds from grapes, obtained by the disruption of the cell wall due to pressure alternance and cavitation (treatment time from 1 to 5 min at three different levels of amplitude − 30, 60 and 90%), with a reduction in the duration of classic maceration. Then, they evaluated its application on lees with the goal of estimating the lytic effect of US on yeast cell structures (1, 3 and 5 min at 30, 60 and 90% of amplitude, with 3 replicates of the test at 60% for 3 min), in order to accelerate the process of aging on lees (Ferraretto et al., 2013).

Cacciola, Batllò, Ferraretto, Vincenzi, and Celotti (2013) tested US effects (treatment time from 1 to 5 min at three different levels of amplitude 30, 60 and 90%) on wine lees, in order to verify the possibility of accelerating the protective colloids (i.e., polysaccharides) release. These effects were compared with the usual practices of aging wine over lees and with the enzyme treatment of lees by means of beta-glucanases able to demolish the glucans and facilitate the release of intracellular components. US treatment on wine lees resulted in an increase in total colloids, proteins, and polysaccharides from yeast in the medium with a reduction in the colloids particle diameter; therefore, a significant effect on the extraction of cellular components compared to the long stay on the lees was detected (Cacciola et al., 2013).

During the stage of oaking, the wine comes into contact with the wood of the barrels (traditional oaking) or with added wood chips, wood sticks/staves or oaking powder (alternative oaking). In this period wine flavor and bouquet gains vanilla, caramel, cream, spice or earthy notes due to the phenols contained in the oak; moreover, the ellagitannins (hydrolyzable tannin), which are formed from lignin structures in wood, play a very important role because they protect the wine from oxidation and reduction. US treatment during wine oaking might be useful thanks to the penetration of liquid into the wooden structure of powder, chips, sticks or staves and the consequent mass transfer that entails a shorter oaking period and better results in terms of flavor and reduction of astringent tannins (Martín & Sun, 2013). Then, by using 25-kHz ultrasound waves to intensify the mass transfer of phenolics from oak chips into a model wine, Tao, Zhang, and Sun (2014a, 2014b) showed that the initial release rate and effective diffusion of total phenolics generally increased with acoustic energy density and temperature (Tao et al., 2014a, 2014b).

To accelerate wine maturation, low frequency ultrasonic waves of 28 and 45 kHz were used to treat the steeped greengage wine. The contents of total acid, total ester, fusel oils, and the wine chromaticity were determined before and after the ultrasonic treatment. The volatile compounds were analyzed by gas chromatography–mass spectrometry (GC–MS) method, and the sensory quality was evaluated by panelist. The results indicated that ultrasonic treatment of the steeped greengage wine at 45 kHz and 360 W for 30 min was effective to accelerate the aging process, where the fusel oils and alcohol compounds were significantly reduced and acid and ester compounds were significantly increased (Zheng, Zhang, Fang, & Liu, 2014).

3.1.1.2. Effect on wine quality. Three measurable parameters are usually responsible for wine quality evaluation: total phenolic compounds, generally related to the organoleptic properties and stability of wines; anthocyans, which are responsible for the color in red wines; and total tannins which account for the astringency and body of wine, as well as they are involved in co-pigmentation and co-polymerization



Scheme 1. Basic steps of red wine production.

reactions with anthocyanins to form more stable structures against the action of sulphur dioxide and oxygen (Dipalmo, Crupi, Pati, Clodoveo, & Di Luccia, 2016).

US treatments have the capacity to increase the amount of phenolic compounds in red wine (Tudose-Sandu-Ville et al., 2012; Coletta et al., 2013) and to accelerate its aging by promoting the polymerization and co-polymerization of anthocyanins and tannins as the wine matures, even though high sound pressures should be avoided because they could cause instead the polymerization suppression (Masuzawa, Ohdaira, & Ide, 2000). Moreover, the positive effect of US treatment (at 35 KHz) on the phenolic extraction from grape skins is completely lacked if must is over treated (15 min of ultrasound maceration), leading, on the contrary, to obtain wine poor in anthocyanins and tannis (Tudose-Sandu-Ville et al., 2012).

Tedjo, Taiwo, Eshtiaghi, and Knorr (2002) studied the quality attributes of grape juices for wine-making using non-thermal processes including US. Quality analyses (e.g. sugar, anthocyanins and mineral concentration, acidity, color) showed that US processed juices had superior quality respect to untreated samples and comparable quality to enzyme treated juices (Tedjo et al., 2002).

US can also be used to improve the extraction of aromatic chemicals, which impart bouquet to the wines (Cocito, Gaetano, & Delfini, 1995). It was emphasised that US improved extraction efficiency with increased reproducibility of most aroma compounds compared to conventional extraction method (Vila, Mira, Lucena, & Recamales, 1999). Regarding the maceration on the skins of white grapes, the cold soaking is often used for aroma purposes since it encourages the extraction of precursor aroma compounds; in this sense, US has been proved to compensate for a reduced enzymatic activity caused by lower operating temperatures improving the extraction of aromatic precursors and compounds during maceration on the skins (Cui et al., 2012).

3.1.1.3. US could replace the preservatives (SO_2) addition during winemaking. It was shown that US treatments have antimicrobial effects (Zenker, Heinz, & Knorr, 2003). Microbial cell inactivation is generally due to three different mechanisms: cavitation, localized heating, and free radical formation. The shear force is one of the modes of action, which leads to disruption of the microbial cells; the pressures produced during bubble collapse are sufficient to break cell wall structures, leading to their leakage and disruption, moreover the localized (near to the cells) and transient high temperatures can provoke thermal damages, such as denaturation of proteins and enzymes.

Of course, bacterial cells are differently sensitive to US treatment depending on their dimension and shape (Knorr, Zenker, Heinz, & Lee, 2004). In general, larger cells are more vulnerable to the high pressures produced during ultrasonication due to their higher surface area (Earnshaw, 1998); Gram positive cells appear to be more resistant to ultrasound than Gram negative one due to the structure of the cell walls and spherical-shaped cells (cocci) are more resistant to ultrasound than rod-shaped one. Moreover, spores have been found to be more resistant to sonication than vegetative bacteria and many microorganisms resistant to heat are similarly resistant to ultrasound (Leadley & Williams, 2006).

With regard to wine microbiota, besides the positive presence of the selected yeasts and lactic acid bacteria involved in alcoholic and malolactic fermentations, the growth of unwanted microorganisms including yeasts (e.g. *Brettanomyces* spp.), lactic acid bacteria (e.g. *Lactobacillus* spp.), and acetic acid bacteria (e.g. *Acetobacter* spp. and *Gluconobacter* spp.), which can lead to the wine spoilage giving excessive bitterness or buttery characters, and higher volatile acidity, should be controlled (Buglass, 2011). In this sense, the use of traditional antimicrobial treatments such as heat, chemical additives (such as SO_2) or sterile filtration are still widespread, but may be restricted by regulations or even be undesirable due to their adverse sensory impacts on the product (Jiranek, Grbin, Yap, Barnes, & Bates, 2008).

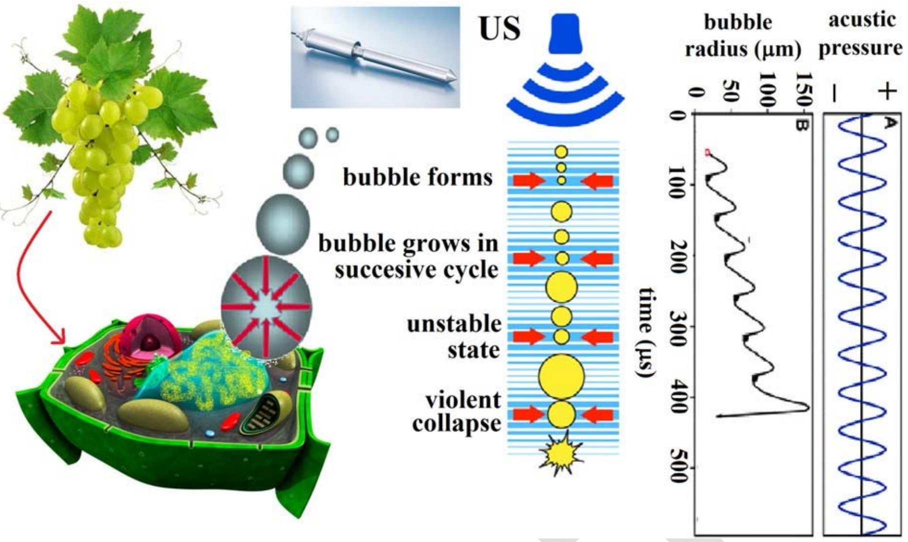


Fig. 1. Ultrasound cavitation phenomena.

For these reasons, Jiranek et al. (2008) proposed high power US as a novel alternative tool offering new opportunities for controlling wine microbiota. In particular, the authors applied US at different stages of the winemaking: i) before the alcoholic fermentation, in order to control wild yeasts (of the genera *Saccharomyces*, *Kloeckera*, *Hanseniaspora*, *Metschnikowia*, *Candida*, *Pichia*, *and Zygosaccharomyces*) as well as the Gram-positive lactic acid and Gram negative acetic acid bacteria; ii) after the primary fermentation, aiming at suppressing the spontaneous malolactic fermentation (that is not required for some products, such as the major part of white and rosè wines) and the growth of spoilage yeasts and bacteria during the further steps of wine maturation and stabilization (Jiranek et al., 2008).

During *sur lee* aging, the lees (mainly consisting of dead yeast cells and small grape particles that accumulate during fermentation) cells break down into simpler constituents, releasing sugars and proteins as well as flavor and aroma compounds that interact with the wine chemistry, positively affecting its overall quality (Patynowski, Jiranek, & Markides, 2002). However, an excessive contact time *sur lee* can generate off flavors (Patynowski et al., 2002); in this sense, US, under controlled conditions, can increase the rate of yeast cell disruption and autolysis, but minimizing the contact time *sur lee*, with positive effects on wine flavor and aroma (Jiranek et al., 2008).

As mentioned before, SO_2 is widely used as additive during the winemaking process (from must pressing to wine bottling) in order to protect wine (especially white wine) owing to its antimicrobial and antioxidant activities. However, sulfites have been associated with the triggering of asthmatic responses in certain individuals and they may cause negative health effects (i.e. migraines and headaches) (Pati et al., 2014). Besides the antimicrobial effects, the possibility to use ultrasound treatments instead of SO_2 addition is strengthened by the capability to inhibit enzymes, such as polyphenol oxidase (PPO), that was investigated by O'Donnell, Tiwari, Bourke, and Cullen (2010). Nevertheless, the application of ultrasound technology still requires a practical evaluation of the feasibility to completely replace the use of SO_2 in winemaking.

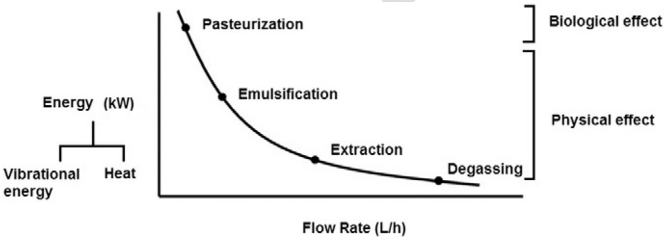


Fig. 2. The conversion of electrical energy into ultrasonic effects: relationship between flow rate (L/h) and energy (kW).

3.1.1.4. Effect of US energy input on the extraction of phenolic compounds. Phenolic compounds are of particular interest in wine industry as it gives the characteristic color and flavor in the wine. Moreover, phenolic compounds (i.e. flavonoids and non-flavonoids) of grape are known for their health-promoting effects (Dragsted, Strube, & Larsen, 1993; Carrieri et al., 2013; Krishnaswamy, Orsat, Gariépy, & Thangavel, 2013).

The beneficial effects of US treatment on the extraction of phenolic compounds and anthocyanins from grape and berry matrix was investigated using an ultrasonic processor UIP2000hd after mashing and enzyme incubation; the disruption of the cell walls by enzymatic treatment (Pectinex BE-3L for bilberries and Biopectinase CCM for black currents) was improved when combined with US treatment with an increase (15–25%) of the phenolic compounds concentration in the juice (Mokkila, Mustranta, Buchert, & Poutanen, 2004).

Chang and Chen (2002) have studied the application of high US frequency (20 kHz) to accelerate the aging of rice wine; they have observed that the amount of acetaldehyde decreases while other pleasant flavor compounds become more prominent thus giving a better flavor to the treated rise wine. Similarly, Zheng et al. (2014) observed that ultrasonic treatment of the steeped greengage wine at the higher frequency (28 and 45 kHz) was effective to accelerate the aging process, where the fusel oils and alcohol compounds were significantly reduced and acid and ester compounds were significantly increased (Zheng et al., 2014).

Tiwari, Patras, Brunton, Cullen, and O'Donnell (2010) have examined the effect of high frequency (20 kHz), amplitude level (24.4–61 lm), and treatment time (2–10 min) on the US extraction of anthocyanins and color in red grape juice; the anthocyanins significantly influenced by both factors investigated were cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and malvanidin-3-*O*-glucoside (Tiwari et al., 2010).

3.1.1.5. Effect of US energy input for the valorization of wine by-products. Grape marc and pomace represent the solid residues (i.e. skins, seeds, and small amount of leaves) after winemaking. It has been reported that million tons of grape marc are produced from the wine industry every year (Monrad, Howrd, King, Srinivas, & Mauromoustakos, 2010; Kammerer, Kammerer, Valet, & Carle, 2014). Grape marcs are usually discarded as natural waste or mostly utilized for alcohol production and tartaric acid extraction; more recently, there is an increasing interest in their use for the recovery of phenolic compounds which can be potentially used in the food industry, thus generating economic benefits and reducing the environmental impact (Vilkhu et al., 2008). Tao et al. (2014a, 2014b) studied the influence of acoustic energy density and temperature during the ultrasound-assisted extraction of phenolic compounds from grape marc. They concluded that ultrasound has a potential benefit in the extraction and isolation of novel potentially bioactive components, e.g. from non-utilized by-product streams formed in winemaking processes. (Tao et al., 2014a).

Drosou, Kyriakopoulou, Bimpilas, Tsimogiannis, and Krokida (2015) compared the efficiency and selectivity of ultrasound assisted extraction (UAE) with the conventional Soxhlet extraction (SE), in terms of extraction yield, antiradical activity (A_{AR}) and total phenolic content (TPC), on dehydrated Agiorgitico red grape pomace by-products. UAE water/ethanol extracts were found to be richer in phenolic compounds (up to 438,984 ± 4034 ppm GAE in dry extract) with higher A_{AR} (0.91 ± 0.02 mg/mL) (Drosou et al., 2015). US can also be employed to extract non-phenolic valuable compounds from wine by-products. In particular the extraction of enzymes and proteins stored in cells and subcellular particles is a unique and effective application of high-intensity ultrasound (Tiwari et al., 2009), as the extraction of organic compounds contained within the grape seeds by a solvent can be significantly improved. Regarding the seed treatment, US can be used to improve the extraction of lipids and proteins. In this case, the destruction of the cell walls facilitates the pressing (cold or hot) and thereby reduces the residual oil or fat in the pressing cake.

Palma and Barroso (2002) optimized the US treatment conditions for the recovery of tartaric and malic acid from red and white variety grapes by-products. Vilkhu et al. (2008) studied on US of tartaric esters from red grape marc founding a yield increase from 16 to 23% using two different varieties. The recovered tartaric acid can be used in bakery operations, wine production, pharmaceutical industry, hardening of gypsum, confectionery processing and in the chemical industry (Vilkhu et al., 2008).

3.1.1.6. Effect of US energy input in process development and scale up. Unlike other non-thermal processes, such as high hydrostatic pressure, compressed carbon dioxide and supercritical carbon dioxide, and high electric field pulses, ultrasound can be easily tested on bench-top scale, generating reproducible results for scale-up. Two main variables can influence the US process: the intensity and the frequency of the waves. US intensity is the power dissipated per unit of surface area (W/cm^2) of the sonotrode (Patist & Bates, 2008); in general, an increase in intensity increases the sonochemical effect De Castro, & Priego-Capote, 2007). US frequency is the number of cycles per unit of time; if frequency increases, the duration of low pressure phase shortens and intensity of ultrasound has to be increased to maintain an equivalent amount of cavitation energy in the system.

The intensity of sonication is proportional to the amplitude of vibration of the ultrasonic source and, as such, an increment in the amplitude, as well as minimum intensity, increases sonochemical effects also into the grape must. The intensity and the cavitation characteristics can be easily adapted to the specific extraction process to target objectives. Amplitude and pressure can be varied in a wide range, e.g. to identify the most energy efficient extraction setup.

The design, geometry, and method by which the ultrasonic transducer is inserted or attached to the reaction vessel is essential to its effectiveness and efficiency, and the right choice of these parameters is mandatory for the industrial up-scaling of the laboratory ultrasound process because any difference between laboratory and inappropriate pilot plant design can often lead to very different results (Leonelli & Mason, 2010).

In general, the system used to apply power ultrasound to grape, must, wine or grape by-product consists of three basic parts:

1. Generator: an electronic or mechanical oscillator that needs to be rugged, robust, reliable, and able to operate with and without load.
2. Transducer: a device for converting mechanical or electrical energy into sound energy at ultrasonic frequencies.
3. Coupler: the working end of the system that helps in transferring the ultrasonic vibrations to the substance being treated (usually liquid).

Convenient measurement and modelling of the pressure and cavitation bubble fields are the central problem. Complete models are extremely complex due to the fact that high acoustic intensities generate cavitation and thus oscillating bubbles. Such bubbles will modify pressure wave propagation through the sonicated fluid and also cause damping. With a heterogeneous system, e.g. one involving suspended solid particles, the acoustic field is even more complex. Despite these difficulties a number of larger scale sonochemical reactors have been developed (Leonelli & Mason, 2010). At commercial level, the company Hielscher Ultrasonics (Teltow, Berlin, Germany) realized different ultrasonic solutions for winemaking application. In particular, for large volume reactor they suggest to employ a titanium probe with the following characteristics: 4000 W and 20 kHz with a processing capacity from 0.1 to 0.8 m^3/hr. The ultrasonic transducer is located in a double-walled stainless steel cabinet that comes with a very effective sound insulation in order to protect the operators into the winery.

3.2. Principles and mechanism of MW

MW are non-ionizing electromagnetic waves of frequency between 300 MHz and 300 GHz (Letellier & Budzinski, 1999); even though, only a few frequencies are allowed for industrial, scientific, and medical uses (ISM frequencies), generally comprised between 0.915 and 2.45 GHz (Chemat & Cravotto, 2012). Overall, MW is a technology applied in many food processes to reduce processing time (Thostenson & Chou, 1999), however its use in food preservation (due to the direct antimicrobial effects on microorganisms or to the possibility to promote the synthesis of a large number of antimicrobial compounds) is largely proposed by the recent scientific literature (Gould & Grahame, 2012; Puttaraju et al., 2013; Pansare, Mulla, Pawar, Shende, & Shinde, 2014).

The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomena: ionic conduction and dipole rotation, which in most cases occurs simultaneously. Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field; the resistance offered by the solution to the migration of ions generates friction, which eventually heats up the solution. While, dipole rotation deals with a re-alignment of the polar molecules (from solvent or target material) with the rapidly changing microwave electromagnetic field, transforming MW energy into heat (Letellier & Budzinski, 1999; Letellier, Budzinski, Charrier, Capes, & Dorthe, 1999). Therefore, MW heating is the conversion of electromagnetic energy to thermal energy through direct interaction of the incident radiation with the molecules of the target material. (Gabriel, Gabriel, Grant, & Halstead, 1998; Klán, Literák, & Relich, 2001; Hoz, Diaz-Ortiz, & Moreno, 2005). The rate of this conversion is describe by Chen, Zhao, Lee, and Liu (2005):

$$P ¼ Kf\varepsilon^0 E^2 \tan\delta$$

where P is the microwave power dissipation per volume unit, K is a constant, f is the frequency applied, ε' is the absolute dielectric constant of the material, E is the electric field strength, and δ is the dielectric loss tangent (Chen et al., 2005). Then, as MW is applied on biological material (such as grape samples), electromagnetic waves are absorbed selectively by media possessing a high dielectric constant, resulting in more effective heating. The increased temperature can overcome the solute-matrix interaction caused by van der Waals forces, hydrogen bonding, dipole attraction of the solutes molecules, and active sites in the matrix. Thus, the supplied thermal energy can disrupt cohesive (solute-solute) and adhesive (solute-matrix) interactions, providing the necessary activation energy required for desorption process. The transfer of the molecules from matrix to solvent is also achieved by the diffusion and convection processes, indeed, after penetrating, MW causes the explosion of the grape cells, quickly releasing their content into the liquid phase (Fig. 3). When the liquid absorbs the MW, the kinetic energy of its molecules increases together with the diffusion rate (Mandal, Mohan, & Hemalatha, 2007).

When used for extractive purpose, the main factors affecting MW are: solvent type and volume, extraction time, microwave power, matrix characteristics, and temperature. A correct choice of solvent, based on its dielectric properties, is fundamental for obtaining an optimal extraction process; the solvent type for MW is dictated by the solubility of the target analyte (i.e. plant matrix) and its capacity to absorb microwaves (Letellier et al., 1999; Mandal et al., 2007). Volume of the extracting solvent is also a critical factor; typically, it should be sufficient to ensure the complete matrix immersion during the entire irradiation time. Conversely, a higher ratio of solvent volume to solid matrix may be more effective in conventional extraction methods, but it may yield lower recoveries with MW technique due to inadequate stirring of the solvent by microwaves (Wang & Weller, 2006). Thus, a careful optimization of this parameter is of primary importance in MW (Mandal et al., 2007).

With regard to the extraction time, generally, its increasing enhances the quantity of analytes recovered, although there is the risk that degradation may occur; indeed, microwave assisted extraction (MAE) of polyphenols and caffeine was found to increase up to 4 min and later decreased with the extraction time (Pan, Niu, & Liu, 2003). Overall, 15–20 min represents an optimal extraction time, but even 40 s have been demonstrated excellent recoveries (Li, Chen, Zhang, & Yao, 2004; Wang et al., 2007). Microwave power and irradiation time are two such factors, which influences each other to a great extent. A combination of low or moderate power with longer exposure may be a wise approach. The power must be chosen correctly to avoid excessive temperature, which could lead to solute degradation and overpressure inside the vessel (Mandal et al., 2007).

Also the matrix characteristics are important, indeed its particle size and status can have a profound effect on the recovery of compounds in MAE. The particle sizes of the extracted materials are generally in the range of 100 μm–2 mm (Wang & Weller, 2006). Fine powder and particles can enhance the extraction by providing larger surface area, which permits a better contact between matrix and solvent with a deeper MW penetration (Mandal et al., 2007).

3.2.1. MW in red winemaking process

3.2.1.1. Effect of MW on reducing winemaking time and aging. In processing of grapes, MW technology can be efficiently used to reduce winemaking time and enhance the overall quality of wine

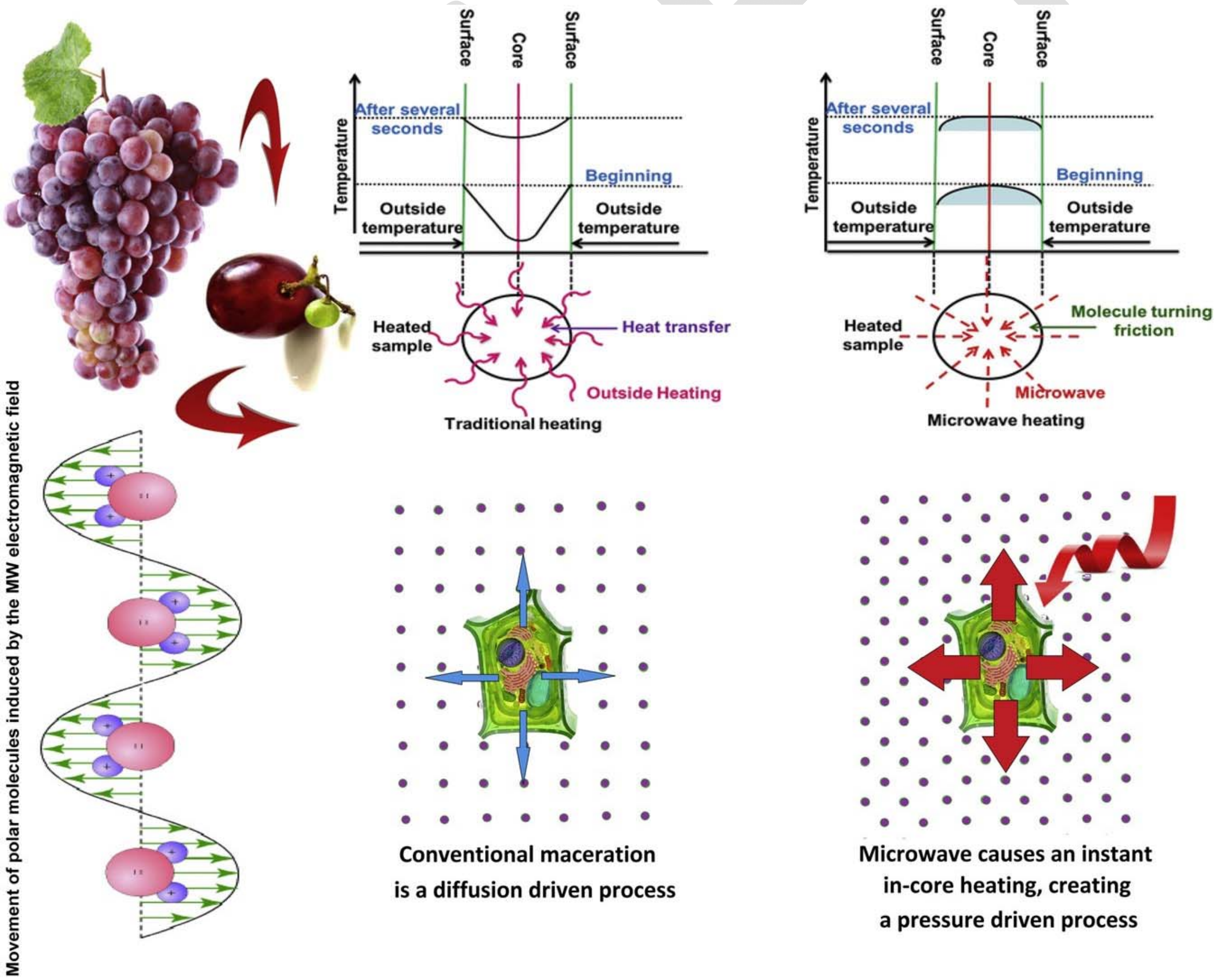


Fig. 3. Microwave cellular effect on grape maceration during winemaking.

(Thostenson, & Chou, 1999). Carew et al. (2013) demonstrated that MW maceration, applied to Pinot Noir grapes, determines a rapid extraction of phenolic compounds into juice respect to the long maceration time of the control and allows to conduct the alcoholic fermentation without pomace, thus avoiding the cap management. Moreover, they observed that MW treated samples showed significantly higher levels of fruity and floral aroma respect to the control, maybe due to the heat inactivation of aroma degrading enzymes and transferases but also to the absence of that volatilization of aroma compounds, typical occurring during cap management (Carew, Close, & Dambergs, 2013a). The same authors, considering that Pinot noir grapes have a unique phenolic profile which can impinge on the extraction and stabilization of compounds such as anthocyanins and tannins, demonstrated that MW maceration treatment favored the extraction of polyphenols from grape solids into grape juice, providing significantly higher concentrations of total phenolics, anthocyanin, tannin, and pigmented tannin (0.60 g/L) in wine at 18 months of bottle age, compared with control wine (0.14 g/L). Moreover, MW treatment was also associated with a substantial and rapid decrease in the grape-endogenous yeast population and a shorter lag phase at the outset of alcoholic fermentation (Carew, Sparrow, Curtin, Close, & Dambergs, 2014).

Zheng, Liu, Huo, and Li (2011) proposed an instantaneous MW irradiation method to treat young dry red wine in order to accelerate the desired aging process during storage of wine for the improvement of its taste quality. They investigated the effects of the microwave power and irradiation duration on the wine sensory property and found that MW treatment had a significant effect on taste and total score, but no obvious effect was found on appearance, fragrance, persistence, and overall assessment (Zheng et al., 2011).

3.2.1.2. Effect of MW on wine quality. Carew et al. (2013) proposed a controlled release of Pinot noir phenolics by treating the grape must with MW. They found that this approach produced Pinot noir wines with richer color and a higher tannin concentration, compared with wines made using a standard submerged cap fermentation process. In the treated wines, anthocyanins and tannins were, respectively, around twice and three folds more concentrated than the control; a more pronounced varietal fruit and floral nose as well as a more soft taste with lovely mouth-coating tannins were also found in the wines after MW maceration (Carew, 2013; Carew, Close, & Dambergs, 2013).

Carew et al. (2013) tested the microwave macerated must and assessed wine phenolics after two different hold times in a 70 °C water bath (1 and 8 h). As regards total phenolics and total and pigmented tannin, both long and short time wine was equivalent to control; conversely, for mean concentration of total pigment, free anthocyanin, and color density, only the short hold time wine was significantly lower than control. However, they underlined that the main result of this MW treatment was the possibility to eliminate laccase and better manage phenolic outcomes with evident benefit for the wine industry (Carew, Connew, Close, & Dambergs, 2013).

3.2.1.3. MW could replace the preservatives (SO_2) addition during winemaking. MW treatment is able to destroy wild microorganisms dangerous for the wine quality, nowadays controlled by means of SO_2 addition to the must or into vats. Carew, et al. (2013) reported a pasteurising effect of MW applied at grape crushing, thus inoculating the selected starters for carrying out the fermentation without the addition of SO_2 (Carew, 2013; Carew, Close, & Dambergs, 2013a). Thanks to the antimicrobial effects, MW can also be potentially used for sanitizing wine vessels and barrels; indeed, González-Arenzana et al. (2013) proposed a novel MW technology application based on the use of a new prototypal equipment, which exploits high frequency microwaves to reduce microbial population in oak wine barrels. They observed a reduction by 36–38% for total yeasts (35 to 67% for *Brettanomyces*) and around 91–100% for lactic acid bacteria and acetic acid bacteria, using a very short treatment time (3 min). Findings from this research suggested that MW would be beneficial for wine industry and environment by increasing barrel functionality, reducing frequency of replacement, improving microbiological control of oak wood, as well as minimizing of preservative use (González-Arenzana et al., 2013).

3.2.1.4. Effect of MW energy input on the extraction of phenolic compounds. In MW technique, the high extraction yield may be the result of a synergistic combination of two transport phenomena: heat and mass gradients, working in the same direction. Moreover, a series of steps are involved during the interaction between the solid parts and the grape must, including (1) penetration of the alcohol/water mixture into the solid matrix; (2) solubilization of phenolics, anthocyanin, and tannin; (3) transport of the phenols out of the skins and seeds; (4) migration of the extracted polyphenols from the external surface of the solid into the bulk solution; (5) movement of the extracted substances with respect to the solid; and (6) separation of the wine and grape pomace. Since rapid breakdown of cell wall takes place at high power (as a result of a higher temperature), often leading to grape must degradation, a combination of low or moderate power with longer contact time may be a wise approach to favor the optimal polyphenols extraction. Indeed, Krishnaswamy et al. (2013) showed that the total phenols level increased, by decreasing the solvent concentration from 60 to 30% and by enhancing the contact time and MW power from 100 to 170 W; the optimum extraction condition was found at 170 W, 6 min, and 30% ethanol concentration yielding 13.5 ± 0.48 mg GAE/g of grape seed dry weight (dw GS) with a desirability of 0.982. MW power of 168 W, 6 min, and 30% ethanol concentration yielded more total phenols in terms of catechin equivalents at 22 ± 0.86 mg CAT/g dw GS with the desirability of 0.997, whereas the predicted optimum extraction conditions, in terms of tannic acid equivalents, were 160 W, 6 min, and 33.7% ethanol concentration with 0.992 desirability giving 16.1 ± 0.56 mg TAE/g dw GS (Krishnaswamy et al., 2013). Moreover, Wang, Xi, Zheng, and Miao (2010) reported that 70% ethanol concentration, microwave power at 255 W, and extraction time of 6.5 min gave maximum flavonoids from *Radix puerariae* (Wang et al., 2010), meaning that future works could be necessary to find out the best MW power according to different grape varieties.

3.2.1.5. Effect of MW energy input for the valorization of wine by-products. Grape pomace is characterized by high contents of bioactive phenolics due to an incomplete extraction during the winemaking process. Therefore, they constitute an inexpensive source for the extraction of phytochemicals that can be used in the pharmaceutical, cosmetic, and food industries. As a result of the increased attention to sustainability of agricultural practices, efforts have been made to use grape pomace in different fields of industry. Thus, it is necessary to have efficient extraction techniques to achieve good recoveries of compounds, such us MW (Kammerer et al., 2014).

Medouni-Adrar et al. (2015) compared the optimized MAE to conventional solvent extraction (CSE) of phenolic compounds from seeds and skin by-products of grape *Vitis vinifera* cv. *Ahmar Bou-Amar*, based on the total phenolic compounds (TPC) and the antioxidant activity. A selective efficiency of the two processes towards the polyphenol extraction from the two grapes part was determined by the authors. Indeed, the TPC of seeds extract obtained with MAE was 24% lower than that of CSE extract and consequently the antioxidant activity of the latter was better than that of the former extract; conversely, the TPC of skin extract obtained with MAE together with its antioxidant capacity was 28% higher than CSE one (Medouni-Adrar et al., 2015).

Casazza, Aliakbarian, Mantegna, Cravotto, and Perego (2010) studied the quali-quantitative optimization of polyphenol extraction from grape wastes using non-conventional extraction techniques, high pressure and temperature extraction (HPTE) and MAE, compared to solid-liquid extraction in term of extraction yield and antioxidant power of the extract. Better findings were gathered using innovative processes; in particular, they found that the highest content in total polyphenols, *o*-diphenols, and flavonoids, both for seeds and skins, was obtained with HPTE, whilst the highest antiradical power was determined in seeds extracts from MAE. Furthermore, MAE was revealed as the more effective technique for *trans*-resveratrol extraction (Casazza et al., 2010).

3.2.1.6. Effect of MW energy input in process development and scale up. Moving from laboratory-scale device (small quantities of compounds) to industrial-scale equipment (enormous quantities of compounds) can be very difficult by using MW technology; indeed, as the scale becomes larger, the advantages of MW can be lost, mainly because the microwave power dissipates along the major distance into the reactor (penetration depth) and this would result in significant overheating at the surfaces, just as with conventional heating. Then, materials that are used for small scale reactions are often not suitable to fabricate larger reactors, and problems of measurement and control can be more acute as the scale of operation is increased.

For larger scale processes, continuous flow reactors are needed; indeed, improved color, flavor, texture, and nutrient retention have been obtained by heating food products using continuous flow MW systems (Giese, 1992; Nikdel, Chen, Parish, Mackellar, & Friedrich, 1993; Villamiel, López-Fandiño, & Olano, 1996; Tajchakavit, Ramaswamy, & Fustier, 1998; Coronel, Simunovic, & Sandeep, 2003; Gentry & Roberts, 2005). However, also the scale-up of a continuous flow microwave system is associated with certain issues, such as a non-uniform temperature distribution within the product because of differences in dielectric and thermophysical properties, non-uniform distribution of electromagnetic field, and the magnitude of the diameter of the applicator tube. Moreover, the control of processing parameters such as microwave power, flow rate, temperature, and pressure can be critical, too. In order to move towards the commercialization of continuous flow microwave technology for the wine-making process, issues related to scale-up and extended run times still need to be addressed.

3.3. Principles and mechanism of PEF

PEF is an emerging non-thermal food processing technology which is receiving considerable attention because of its potential to enhance conventional processing operations as well as to allow cold pasteurization/sterilization treatment of food (also fresh-like liquid foods) replacing or partly substituting for thermal processes (Raso & Heinz, 2006); of course, foods treated in this way retain their fresh aroma, taste, and appearance with excellent nutritional value and shelf-life (Ramaswamy & Marcotte, 2005).

PEF treatment involves the application of short duration electric field pulses (usually for few microseconds) of high intensity (in the order of 20–80 kV) to materials located into a treatment chamber consisting of at least two electrodes and insulation zone (Ramaswamy, Jin, Balasubramaniam, & Zhang, 2005); exposing a biological cell (plant, animal, and/or microbial) to these conditions induces the formation of temporary or permanent pores on the cell membrane (electroporation), which causes the permeation of small molecules through the cell membrane followed by swelling and its eventual disintegration, if the intensity of the treatment is sufficiently high (De Vito, 2006). Pore formation is reversible or irreversible depending on factors such as the electric field intensity, the pulse duration, and number of pulses (Ramaswamy et al., 2005).

Pulses commonly used in PEF treatments are either exponential or square wave shape (Alvarez, Condon, & Raso, 2006); in the former, the voltage rises quickly to a maximum value and decays slowly to zero, while, in the latter, the maximum electric field remain constant during the duration of the pulse. The processing time or total duration of the treatment is defined as:

$$t ¼ n\tau_p$$

where τ_p is the pulse duration and n is the number of the pulses applied to the food. The equation shows that the treatment time increases either with the number of pulses or with the pulse duration, but it is important to observe that increasing the number of pulses, the total energy consumption increases, while with increasing the pulse duration the temperature of food arises.

As aforementioned, during treatments, the food material is in close contact with the electrodes and, therefore, is subject to an electric field. Assuming the sample homogeneous, the average electric field strength E is as follows:

$$E ¼ V=d$$

where V is the voltage across the sample and d the distance between the electrodes (De Vito, 2006). PEF induces a transmembrane potential difference across the cell membrane. When the potential difference reaches a critical value, called the breakdown potential, localized electrical breakdown of the membrane occurs and cell permeability increases (Zimmerman, Pilwart, & Riemann, 1974). As shown in Fig. 4, the permeabilization of plant cell membrane improves the mass transfer in subsequent processes such as expression, dehydration and rehydration, osmotic dehydration or extraction of plant metabolites (Vorobiev & Lebovka, 2006; Toepfl, Heinz, & Knorr, 2006a).

Besides the electric field, the main process variables affecting PEF include temperature, whose increment across the PEF treatment chambers is due to the delivery of electrical energy, but, generally, ambient, sub-ambient, or slightly above-ambient temperature are reached; pressure, that is applied to inhibit the formation of air bubbles in which electrical arcing could occur with fields above 20,000 V/cm; time of exposure, the field is cycled about 1000 times/s, and the fluid is exposed to multiple pulses by passing it through several treatment chambers; and high power sources, depending on the specified type of voltage wave form as well as on the available elements (capacitors, inductors, transformers, and switches), basic pulsed power circuits, circuits with voltage multipliers, pulse forming circuits or networks with pulse forming switches can be used (Singh & Kumar, 2011).

In contrast to other non-thermal processes, such as high hydrostatic pressure, PEF technology requires very short processing times and the treatment can be applied in continuous flow (Puertolas, Lopez, Condon, Alvarez & Raso, 2010).

3.3.1. PEF in red winemaking process

3.3.1.1. Effect of PEF on reducing winemaking time and aging. PEF is a novel food processing technology that poses a very promising future to the enological field, due to its capacity to improve the mass transfer phenomenon. The continuous development of this technology allows nowadays the application of treatments at the semi-industrial scale (Puértolas, Saldaña, Condón, Alvarez & Raso, 2009). García-López, Canosa, and Rodríguez (2008), in accordance with Puértolas, Saldaña, Condón, Alvarez and Raso (2010), found that PEF treatment previous to the maceration step in the winemaking process of the red wine can contribute to reduce the duration of the maceration during vinification or to increase the quantity of polyphenols in the final wine, particularly interesting when grapes poor in polyphenols are considered. They have examined in Cabernet Sauvignon red wines, during winemaking and aging for 4 months in bottle, that the maceration time for wine obtained from grape pomace treated by PEF was 48 h shorter and color intensity (CI), anthocyanin content (AC), and total polyphenols index (TPI) were higher than the control wine, moreover the differences observed at the end of the alcoholic fermentation have remained during the malolactic fermentation step and maturation process (López, Puértolas, Condón, Alvarez, & Raso, 2008a; Puertolas, Lopez, Condon, Alvarez, & Raso, 2010; Puertolas, Saldaña, Condon, Alvarez, & Raso, 2010).

Puértolas et al. (2010) tested the application of a PEF treatment before maceration for reducing the extraction time and improving the

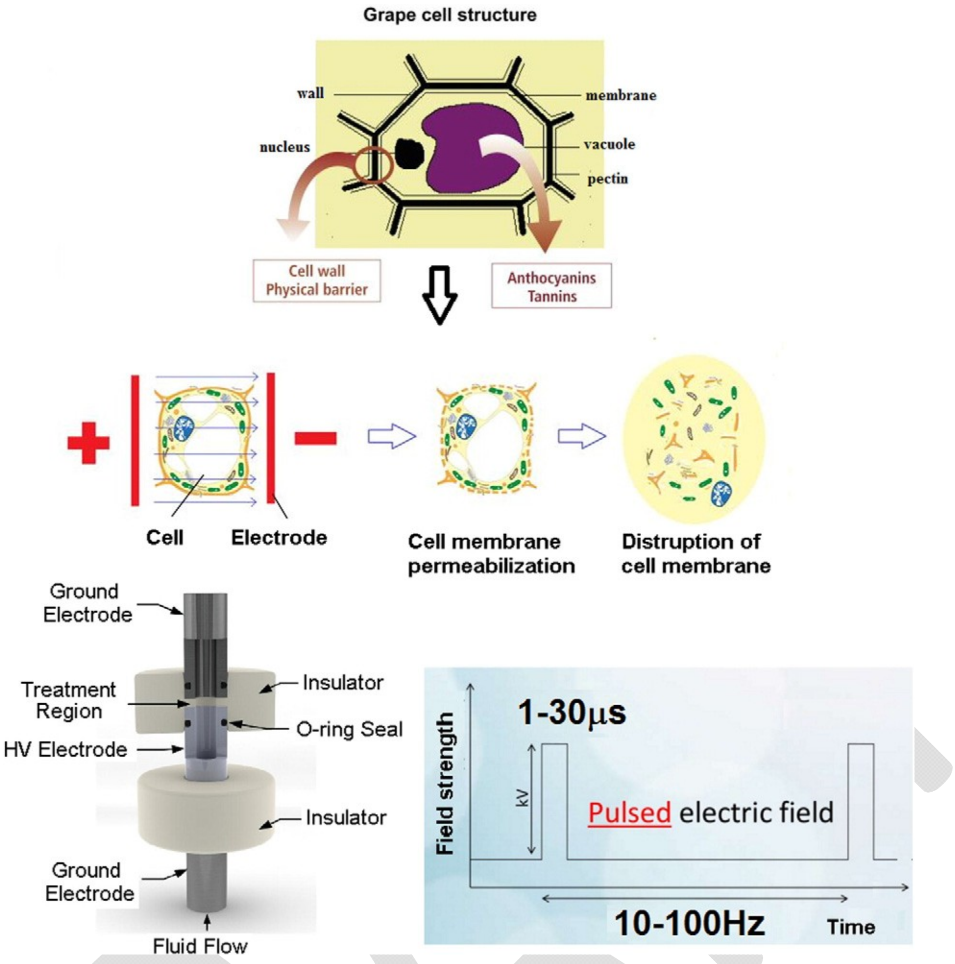


Fig. 4. Mechanism of grape cell membrane rupture by the external applied electric field.

quality of the resulting aged wine; the gathered findings confirmed that the better chromatic characteristics and higher phenolic content were due to the PEF treatment after the fermentation process. These color characteristics were shown to remain or even increase during aging under oxidative conditions, at least if American oak barrels are used (Puértolas, Saldaña, Alvarez, & Raso, 2010).

3.3.1.2. Effect of PEF on wine quality. The quality is an important factor and any processing method applied to wine should not adversely affect on it. The quality of red wine depends on several factors including total phenol content, total antioxidant capacity, total monomeric anthocyanin content, color, aroma, and sensory perception.

The capacity of PEF to inactivate microorganisms and to induce permeabilization of cells without an important increase of the temperature of the product matrix offers potential applications for the winemaking process in order to improve wine quality (Puertolas, Lopez, Condon, Alvarez, & Raso, 2010). García-López et al. (2008) observed that the permeabilization of the Tempranillo grape skin by application of PEF treatments (at 5 and 10 kV/cm) at room temperature caused an increment of the color intensity, anthocyanin content, and total polyphenolic index with respect to the control during all the vinification process. The total phenolic index increased considerably with the application of a PEF treatment at 5 kV/cm, while a further increment on the electric field strength did not appreciably augment this attribute (López, Puértolas, Condón, Alvarez, & Raso, 2008b). A similar behavior was also found in the polyphenols extraction from Graciano and Grenache (López-Giral et al., 2015).

PEF did not affect other wine characteristics such as alcohol content, total acidity, pH, reducing sugar concentration and volatile acidity; Puértolas et al., (2010) observed that the High Performance Liquid Chromatography (HPLC) anthocyanic profiles of freshly fermented model Cabernet Sauvignon wines obtained from PEF-treated pomace were similar to those of the control wine, indicating that the permeabilization of the cell membranes of pomace did not produce a selective effect on any anthocyanin. Malvidin-3-glucoside and malvidin-3-acetylglucoside were the predominant anthocyanins in both the control and the PEF wine. Furthermore, wine obtained from PEF treated grape, from a sensory point of view, was similar to the control wine, without any strange taste or off-flavors (Puertolas, Saldaña, Condon, Alvarez, & Raso, 2010).

The applicability of PEF to process red wine without affecting important quality parameters is very important. Abca and Akdemir Evrendilek (2014) revealed that PEF processing with maximum of 31 kV/cm at 40 ± 2 °C can be successfully used to process red wine samples with no significant change on pH, °Brix, titratable acidity, color, total monomeric anthocyanin content, total phenolic substance content and total antioxidant capacity as well as sensory properties. Moreover, the effect of the addition of two enzymatic preparations and the application of PEF on the phenolic content and color of Cabernet Sauvignon wine has been compared. The evolution of CI, AC, TPI from crushing to three months of aging in bottle was studied. The results demonstrated that both treatments promoted greater extraction of phenolic compounds, compared to the untreated wine; indeed, after 3 months of storage, CI, AC, and TPI were 28%, 26%, and 11%, respectively, higher in PEF wine than in control wine. By contrast, while both enzymatic preparations increased the CI of the wine around 5%, only one of them increased the AC and TPI by 11% and 3%, respectively, in comparison with the control. The content of nonanthocyanic families, determined by HPLC, was higher in PEF-wine; instead, in wines treated by enzymes, only an increase of phenolic acids and flavonols was detected (Puértolas, López, Condón, Raso, & Alvarez, 2009).

3.3.1.3. PEF could replace the preservatives (SO_2) addition during winemaking. During and after the fermentation, wine can be spoiled by different microorganisms which can be part of the natural microbiota of grape skins or derive from contact surfaces, such as from winery equipment and barrels (Couto & Sanromán, 2005). As mentioned before, the addition of SO_2 is the usual practice in wineries to decrease the risk of microbial spoilage during the winemaking; however, PEF technology has been reported as an alternative method for the inactivation of microorganisms in must and wine (Garde-Cerdan, Marselles-Fontanet, Arias-Gil, AncinAzpilicueta, & Martin-Belloso, 2008; López et al., 2008a; López et al., 2008b; Puértolas, López, Condón, Raso and Alvarez, 2009; Puertolas, Lopez, Condon, Alvarez & Raso, 2010). The antimicrobial effect of PEF depends on the cell membrane electroporation leading to the impermeabilization of the membrane to ions and macromolecules (González-Arenzana et al., 2015); Garde-Cerdan et al. (2008) have shown that when the must is treated by PEF, the addition of SO_2 aimed at the control of wild yeasts and bacteria could be reduced, or even eliminated without any relevant effect on the content of volatile compounds of the final product. Indeed, it was confirmed that the absence of SO_2 in these conditions has no negative impact on the sensory characteristics of wine (Garde-Cerdan et al., 2008).

Several investigations were carried out on the effects of PEF treatments on yeasts and bacteria (González-Arenzana et al., 2015), founding a noticeable variability depending on treatment conditions, microbial strain belonging to the same species, and matrix characteristics. Marsellés-Fontanet, Puig, Olmos, Minguez-Sanz, and Martín-Belloso (2009) found that PEF processing at 35 kV/cm provided 4 log inactivation of the yeasts *Kloeckera apiculata* and *Saccharomycescerevisiae*, and different bacteria (*Lactobacillusplantarum*, *Lactobacillushilgardii*, *Gluconobacter oxydans*), while Puértolas, López, Condón, Raso and Alvarez (2009) demonstrated that a treatment with 16–31 kV/cm electric field strength provided 3 log reductions of *Dekkera anomala*, *Dekkerabruxellensis*, *L. plantarum* and *L. hilgardii* in both must and wine (Marsellés-Fontanet et al., 2009; Puértolas, López, Condón, Raso and Alvarez, 2009). Abca and Akdemir Evrendilek (2014) studied the effect of PEF in combination with moderate heat treatment on the inactivation of yeasts (*Candida lipolytica*, *S. cerevisiae*, and *Hansenula anomala*) and bacteria (*Escherichia coli* O157:H7, *Lactobacillus delbrueckii* ssp. *bulgaricus*) founding that high electric field strength (up to 31 kV/cm, at the treatment temperature ranging from 10 to 30 °C) caused different inactivation rates of the microrganisms in wine (*H. anomala* = *C. lipolytica* = *S. cerevisiae* **N** *E. coli* O157:H7 **N** *L. delbrueckii ssp. bulgaricus*).

Overall, the sensitivity of microorganisms to PEF depends on structure and size of the microbial cells, but matrix factors such as water activity, pH, soluble solids, ethanol concentration, and electrical conductivity, markedly affect the treatment results (González-Arenzana et al., 2015; Yang, Huang, Lyu, & Wang, 2016). Also the temperature plays a synergistic role in the inactivation of microorganisms (the lower the inlet temperature is, the higher specific energy is required) (González-Arenzana et al., 2015), although high temperatures cannot be applied to wine without compromising its quality properties. In particular, González-Arenzana et al. (2015) observed that, depending on the PEF treatment parameters (electric field strength, energy, time, pulse width, and temperature), the most sensitive microorganism can belong to any of the acetic acid bacteria, lactic acid bacteria, or yeast groups. Moreover, they reported that it is very difficult to categorize microbial PEF resistance based on size, shape, or membrane structure of the different microbial species encountered in must and wine and, contrarily to some previous findings, they demonstrated that the energy required to inactivate bacteria was not higher than that necessary for yeasts (González-Arenzana et al., 2015).

Delsart et al. (2015) proposed PEF for the microbial stabilization of red wines before bottling, in particular for the inactivation of *O. oeni* CRBO 9304, *O. oeni* CRBO 0608, *Pediococcus parvulus* CRBO 2.6, and *Brettanomyces bruxellensis* CB28. They found that a PEF treatment at 20 kV/cm for 4 ms at low temperature (T ~ 10 °C) was enough to inactivate all microorganisms present in the wine before bottling but without modifying their composition (Delsart et al., 2015).

3.3.1.4. Effect of PEF energy input on extraction of phenolic compounds. The main variables of high-intensity PEF technology are: pulse frequency (Hz), pulse width (μs) and polarity (monopolar or bipolar), flow rate (L/h), pulse shape (exponentially decaying, square-wave, etc.), electric field (kV/cm), and specific energy (kJ/kg). Increasing the electrical field strength, pulse duration, or number of pulses can enhance both the degree of membrane rupture and increase the density (that is, number and size) of pores in the membrane and cell wall (Zimmermann, 1986; Weaver & Chizmadzhev, 1996; Knorr & Angersbach, 1998; Fincan & Dejmek, 2002; Arevalo, Ngadi, Raghavan, & Bazhal, 2003; Bazhal, Ngadi, & Raghavan, 2003; De Vito, Ferrari, Lobovka, Shynkaryk, & Vorobiev, 2008; Asavasanti, Ersus, Ristenpart, Stroeve, & Barrett, 2010). Little is known, however, about the effect of pulse frequency on plant cell membrane electroporation (Vorobiev & Lebovka, 2008). Earlier studies have been carried out primarily with artificial animal membranes, lipid vesicles, microbial, and animal cell suspension cultures or with plant protoplasts. These studies suggest that lower pulse frequencies may cause more damage to the cell because there is more time for charging the cell membranes between pulses, thereby facilitating pore formation (Bruhn, Pedrow, Olsen, Barbosa-Canovas, & Swanson, 1997; Vernhes, Cabanes, & Teissie, 1999; Vernhes, Benichiu, Pernin, Cananes, & Teissie, 2002; Bilska, DeBruin, & Krassowska, 2000; Evrendilek & Zhang, 2005; Loghavi, Sastry, & Yousef, 2008).

García-López et al., (2008) have studied the effect of strength (PEF) treatments (2, 5, and 10 kV/cm) to the grape skin on the evolution of color intensity, anthocyanins, and index of total polyphenols, along the vinification process of three grape varieties (Garnacha, Mazuelo, and Graciano). They observed that the electric field strength between 2 and 10 kV/cm had no significant influence on the values of color intensity, anthocyanins, and total polyphenols of the Garnacha and Graciano wine, indicating a great dependence on the grape variety. However, a statistical significant influence of the electric field strength was always observed when this parameter increased from 2 to 10 kV/cm in Mazuelo wine; in particular, a treatment at 10 kV/cm was more effective than treatments at 2 or 5 kV/cm (López et al., 2008a).

In a study on Tempranillo must and wine, the color intensity and anthocyanin content of the wine were shown significantly increased, up to 28.6% higher than in the control sample, when the PEF treatment was applied at 10 kV/cm and after 96 h of maceration. Even though, the total phenolic index increased considerably also with the application of a PEF treatment at 5 kV/cm (López et al., 2008b). These results confirm the observations of other authors that reported an increment in the extraction efficiency by increasing the electric field strength applied (Jemai & Vorobiev, 2002; Álvarez, Pagán, Condón, & Raso, 2003).

Generally the application of a PEF treatment was more effective at higher electric field strengths, except for some varieties such as Cabernet Sauvignon, for which the most successful treatment was at 5 kV/cm. Then, considering the most effective treatments for each grape variety, the application of a PEF treatment increased the color intensity of the wine between 19 and 61%, the anthocyanins concentration between 18 and 43%, and the total phenol index between 14 and 45% (Puertolas, Lopez, Condon, Alvarez & Raso, 2010; López-Alfaro et al., 2013).

3.3.1.5. Effect of PEF energy input for the valorization of wine by-products. As previously mentioned, grape pomace, as residues of winemaking, are good sources of many phytochemicals known for their antioxidant potential. Therefore, the extraction of bioactive compounds from wine pomace can increase the value of this by-product and generate additional income to farmers and wine makers. (Kammerer, Claus, Carle, & Schieber, 2004). Pulsed electric field treatment is effective in enhancing the extraction of polyphenols from grape pomace and peel.

Khalil (2011), for instance, has studied the effects of the pectinase and pulsed electric field treatments on the extraction of polyphenols from three cultivars of grape pomace and one cultivar of grape peel: PEF showed better results than the pectinase treatment, in term of extraction efficiency, even though, in some cases, extracts of the same grape cultivar showed a different phenolic composition in dependence on the treatment chosen.

Corrales, Toepfl, Butz, Knorr, and Tauscher (2008) analyzed the efficiency of novel extraction techniques, among which PEF, for the extraction of anthocyanins from grape by-products. They highlighted that the application of novel technologies increased the antioxidant activity of grape by-products extracts being the extraction carried out with PEF four-fold higher than the control one. In addition, the extraction of individual anthocyanins was studied showing a selective extraction based on the glucose moieties linked to the anthocyanidins and, in particular, anthocyanin monoglucosides were better extracted by PEF (Corrales et al., 2008).

Brianceau, Turk, Vitrac, and Vorobiev (2015) studied the combination of densification and PEF treatment applied on grape pomace of low relative humidity (~55%), without any addition of conductive liquid, as new process for their valorization. This process allowed an efficient selective extraction of total anthocyanins in a range of temperatures, replacing the conventional pre-treatments of raw material (e.g. dehydration and grinding), which have a negative impact on product quality and energy consumption, which can be advantageous in the industrial implementation of PEF (Brianceau et al., 2015).

Barba, Brianceau, Turk, Boussetta, and Vorobiev (2015) have compared the efficiency of alternative treatments (US, PEF, and high voltage electric discharges - HVED) on solvent-free extraction of high added value components, in particular anthocyanins recovery, from aqueous suspensions of fermented grape pomace. Even though, HVED proved to be the most interesting technique to achieve higher phenolic compounds recovery with lower energy requirement, it was less selective than PEF and US regarding the amount of anthocyanins recovered. Furthermore, at equivalent cell disintegration, PEF remarkably increased the extraction yield of total anthocyanins up to 22 and 55% (Barba et al., 2015).

3.3.1.6. Effect of PEF energy input in process development and scale up. The high intensity PEF processing system is a simple electrical system. Coupled with the treatment chambers, the pulse-forming network can be composed by an electrical circuit consisting of one or more power supplies, with the ability to charge voltages (up to 60 kV), switches (ignitron, thyratron, tetrode, spark gap, semiconductors), capacitors (0.1–10 μF), inductors (30 μH), and resistors (2 Ω–10 MΩ) (Góngora-Nieto, Sepúlveda, Pedrow, Barbosa-Cánovas, & Swanson, 2002). PEF equipments can use ordinary electricity and they meet electrical safety standards without the risk of harmful environmental by-products (Ramaswamy & Marcotte, 2005). PEF is an energy efficient process compared to the traditional thermal processes (Vega-Mercado et al., 1997); a full-scale PEF system can process between 1000 and 5000 L of liquid foods per hour and this technology is easy scalable (Sampedro & Zhang, 2012). Generation of high voltage pulses having sufficient peak power (typically megawatts) is the limitation in processing large quantities of fluid, economically (Gaudreau, Hawkey, Petry, & Kempkes, 1998), but the emerging of solid-state pulsed power systems, which can be arbitrarily sized by combining switch modules in series and parallel, can remove this limitation (Gaudreau, Hawkey, Petry, & Kempkes, 2001).

4. Conclusions and perspectives

Today it is essential to make modifications to traditional practices and adopt novel processing technologies in order to fulfill consumers' expectations towards food products characterized by convenience, variety, adequate shelf life and caloric content, healthy properties, reasonable cost, and environmental sustainability. Industrial food producers, in increasingly competitive markets, have to consider the implementation of traditional processes with emerging technologies in order to increase food safety and the ability to offer better products to consumers and guarantee higher profit for their enterprises, even reducing process time and use of natural resources, such as energy, water, and chemicals. In this context, numerous researches have strongly demonstrated the great benefits of new emerging technologies, such as PEF, US, and MW, into the wine industry. Their application can increase compounds extraction during maceration of the must and/or accelerate the stabilization stage. Therefore, the employment of emerging technologies provides high-quality wines with reduced spoilage organisms, low SO_2 content, and higher content in anthocyanins together with pleasant flavor compounds. Moreover, they could confer added value in terms of nutritional or sensorial characteristics of the resulting wine.

However, further research is needed, since the conditions assayed are still very limited. It might be interesting to assay emerging technologies on different grape varieties, which may react otherwise, and examine the effects of the interaction of these technologies combined each other.

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